A METHOD TO PREVENT SIDE-EFFECTS AND INSENSITIVITY TO THE THERAPEUTIC USES OF TOXINS

Field of the Invention

In general, this invention relates to use of human immune globulin. More specifically, the invention relates to the treatment of disorders for which botulinum toxin is used therapeutically.

Background of the Invention

Therapeutic Use of Botulinum Toxin:

Botulinum neurotoxin is a protein molecule that is produced by the bacterium *Clostridium botulinum*, and it is considered to be the most deadly poison known. (Gill, M.D., "Bacterial toxins: a table of lethal amounts" Microbiol. Rev. (1982) 46:86-94.) *Clostridium botulinum* is the species name assigned to four metabolically diverse groups of anaerobic bacteria whose one common feature is the production of botulinum neurotoxin. Seven different antigenic variants of the botulinum neurotoxin molecule are presently known and are serologically distinguishable from each other by means of monovalent antitoxin antibodies. These different toxin types have arbitrarily been assigned the letters A through G. All known botulinum or botulinum-like neurotoxins have in common the same fundamental structure of a di-chain peptide (after proteolytic cleavage) of approximately 150,000 daltons molecular weight. These neurotoxins cause weakness and flaccid paralysis by blocking the motor nerves at the neuromuscular junction, preventing release of the chemical signal that causes

muscle contraction. Botulinal toxins cause human diseases such as infant botulism, foodborne botulism and wound botulism.

The properties of botulinal toxins have allowed them to be used therapeutically. Botulinum toxin is used to produce a temporary muscle paralysis in diseases characterized by: 1) overactivity of a particular muscle or muscle group (e.g., strabismus); 2) involuntary muscle spasm (the dystonias); and 3) other disorders of movement. Numerous therapeutic uses for botulinum toxin were addressed at a November 1990 National Institutes of Health Consensus Development conference. The consensus panel from this conference resolved that botulinum toxin therapy is safe and effective for treating strabismus, blepharospasm, hemifacial spasm, adductor spasmodic dysphonia, jaw-closing oromandibular dystonia, and cervical dystonia. (Clinical Use of Botulinum Toxin. (*Reprinted from* NIH Conses. Dev. Conf. Consens. Statement 1990 Nov 12-14;8(8)) Because the effects of the toxin last for only a few months, repeated injections of toxin are necessary to sustain its therapeutic benefit for chronic conditions.

For example, in December 1989, the U.S. Food and Drug Administration (FDA) licensed for medicinal use a crystalline preparation of botulinum type A toxin, Oculinum® (Allergan, Inc., Irvine, California). Oculinum® contains botulinum neurotoxin, other bacterial protein molecules that co-crystallized with the neurotoxin, and stabilizing materials. Oculinum® is typically used to treat diseases such as strabismus, blepharospasm, hemifacial spasm, adductor spasmodic dysphonia, jaw-closing oromandibular dystonia and cervical dystonia.

Additionally, a number of organisms producing botulinum-like toxins have been identified. For example,

a unique strain of *Clostridium baratii* produces a type F-like toxin, and a unique strain of *Clostridium butyricum* produces a type E-like toxin.

Neurotoxigenic *Clostridium baratii* was discovered, in 1985, to be the unexpected cause of a case of infant botulism in New Mexico (Hall, J.D., et al., "Isolation of an Organism Resembling *Clostridium baratii* Which Produces Type F Botulinal Toxin from an Infant with Botulism." J. Clin. Microbiol. (1985) 21:654-655); since then, it has been identified as the etiological agent of two cases of infant-type botulism in adults (Hatheway, C.L., "Bacteriology and Pathology of Neurotoxigenic Clostridia" pp. 497-508 *in* DasGupta B.R., ed., Botulinum and Tetanus Neurotoxins: Neurotransmission and Biomedical Aspects, Plenum Press, New York (1993, *in press*)). Neurotoxigenic *Clostridium butyricum* was discovered, in 1986, to be the unexpected cause of two cases of infant botulism in Rome, Italy (Aureli, P., et al., "Two Cases of Type E Infant Botulism Caused by Neurotoxigenic *Clostridium butyricum* in Italy. J. Infect. Dis. (1986) 154:207-211).

The name *Clostridium argentinense* has been proposed for the species presently known as *Clostridium botulinum* type G. (Suen, J.C., et al., "*Clostridium argentinense*, sp. nov: A Genetically Homogenous Group Composed of all Strains of *Clostridium botulinum* Toxin Type G and some Nontoxigenic Strains Previously Identified as *Clostridium subterminale* or *Clostridium hastiforme*." Int. J. Syst. Bacteriol. (1988) 38:375-381)

Initially it was believed that individuals exposed to botulinum toxin did not produce antibodies against the toxin, due to the phenomenal potency of the toxin. It was thought that an immunogenic dose of the toxin would be lethal, i.e., that the amount of toxin needed to induce antibody production exceeded the lethal

dose. This belief derived from decades of experience with foodborne botulism.

Clostridium botulinum and its toxin were first described as the cause of foodborne botulism in 1897. (Van Ermengem E., "Ueber einem neuen anaeroben Bacillus und seine Beziehungen zum Botulismus" Z Hyg Infektionskrankh (1897):26:1-26. English translation, Rev Infect Dis (1979) 1:701-19.) Based on the experience with foodborne botulism, it had been determined that no antibodies developed in patients who survived the illness, even among patients who were so ill that they required mechanical ventilation for survival. (Koenig, M.G., et al., "Clinical and Laboratory Observations on Type E Botulism in Man" Medicine (1964) 43:517-45) Consistent with this understanding, it had been reported that patients who recovered from either type B or type E foodborne botulism later experienced a second occurrence of foodborne botulism caused by the same toxin type. (Beller M., Middaugh, J.P., "Repeated type E botulism in an Alaskan Eskimo" N. Engl. J. Med. (1990) 322:855; Schroeder, K., Tollefsrud, A.L., "Botulism from Fermented Trout" T. Norske Laegeforen (1962) 82:1084-87) These reports were used to support the conclusion that exposure to minute, disease-causing amounts of botulinum toxin did not result in the development of antibodies to the toxin.

The failure of the immune system to make antibodies when exposed to botulinum toxin through illness was considered to be analogous to the experience with the human illness tetanus. Tetanus results from the effects of a neurotoxin (tetanospasmin) produced in infected wounds by *Clostridium tetani*, a member of the same bacterial genus as *Clostridium botulinum*. Of all known toxins, tetanospasmin is second only to botulinum toxin in potency. (Gill, M.D., "Bacterial Toxins: A Table of Lethal Amounts" Microbiol. Rev. (1982) 46:86-94)

Experience with tetanus had shown that "the quantity of tetanospasmin required to produce tetanus is insufficient to induce a protective immune response, and patients with this disease require a primary immunization series." (Mandell, G.L., Douglas, R.G., Bennett, J.E. eds., Principles and Practice of Infectious Diseases, 3d ed., Churchill Livingstone, New York, (1990) at p. 1845). Thus, with botulism as with tetanus, it was understood that an immunogenic dose of toxin exceeded the lethal dose.

However, in the context where botulinum toxin is used therapeutically, a new picture has developed. It has been observed that some patients who initially benefitted from the toxin, later became insensitive (refractory, resistant) to its use. This insensitivity has been attributed to the development, upon repeated injections with the toxin, of antibodies against the toxin.

Evidence that patients were developing neutralizing antibodies against the toxin after repeated treatments, thereby becoming unresponsive to the therapeutic effects of the toxin, began to emerge in the late 1980's. Brin and colleagues in 1988 reported that two of 90 patients they studied had developed antibodies to botulinum toxin and had become refractory to treatment. (Brin, M.F., et al. "Localized Injections of Botulinum Toxin for the Treatment of Focal Dystonia and Hemifacial Spasm" Adv. Neurol. (1988) 50:599-608) Jankovic and Schwartz obtained sera from 14 patients characterized as "non-responders" to botulinum toxin therapy, and found neutralizing antibodies against the toxin in 5 (37.5%); no antibodies were found in 32 patients characterized as "responders" to the toxin ($P<0.0001$). (Jankovic, J., Schwartz, K.S., "Clinical correlates of response to botulinum toxin injections"

Arch. Neurol. (1991) 48:1253-56) The patients with antibodies had, on average, received approximately twice as much toxin [1600 U, range 500-2450] as had the patients without antibodies [891 U, range 100-2150]. Additionally, Scott identified seven dystonia patients who had become refractory to treatment; all had neutralizing antibodies present in their sera. (Scott, A.B., "Clostridial Toxins as Therapeutic Agents" pp. 399-412 in Simpson, L.L., ed. Botulinum neurotoxin and tetanus toxin, Academic Press, NY (1989)) Six of the patients in the Scott study had received 300-400 ng and one only 100 ng of toxin within a 30-day period. (For clinical purposes, 1 ng of Oculinum® equals approximately 2.5-3.0 U.)

In England, Hambleton and colleagues studied 20 patients categorized as "maintained response" or as "diminished response." (Hambleton, P., Cohen, H.E., Palmer, B.J., Melling, J., "Antitoxins and botulinum toxin treatment" Brit. Med. J. (1992) 304:959-60 at 959) These patients were selected from a group of several hundred spasmodic torticollis patients who had been treated for several years with botulinum type A toxin. Seven (35%) of the patients studied were found to have toxin-neutralizing antibodies that considerably diminished or abolished their therapeutic response to the toxin.

The American and British findings are especially notable when taken together, since the British investigators used a preparation of botulinum toxin that was made in England, in contrast to the preparation that is both made and used in the United States. Hence, neutralizing antibodies have arisen in patients irrespective of whether the British or American preparation of botulinum toxin was used.

Heretofore, the research emphasis concerning the therapeutic use of botulinum toxin has focused on development of more highly purified toxins as a means to control the immune response.

The focus on developing more highly purified toxins has been noted by two editorials from late 1992, editorials that overviewed the therapeutic use of botulinum toxin. One editorial appeared in the December 19/26, 1992 issue of the Lancet ("Botulinum Toxin" Lancet (1992) 2:1508-9), and the other was published November 14, 1992 in the British Medical Journal (Lees, A.J., "Botulinum Toxin: Useful in Adult Onset Focal Dystonias" BMJ (1992) 305:1169-70 at p.1170).

The Lees editorial, noted that "[p]atients have continued to respond with benefit for more than five years, although antibodies to the toxin may develop in the peripheral blood, leading to initial unresponsiveness or late resistance (Lees, A.J., "Botulinum Toxin: Useful in Adult Onset Focal Dystonias" BMJ (1992) 305:1169-70 at p. 1170). The Lees editorial concluded stating, "Trials of other types of botulinum toxin are under way, and more effective toxins capable of producing longer durations of benefit without inevitably increasing unwanted effects may be developed in the near future." Thus, the proposed solution to the problem of antibody formation and resultant insensitivity was to increase the purity of the toxins used or to develop the other botulinum toxin serotypes (e.g., B, C, D etc.).

The Lancet editorial states "Although the toxin moiety itself is known to be antigenic, toxin neutralising antibodies could also arise from other parts of the BtA-hemagglutinin complex, so a different preparation of BtA [botulinum toxin type A] might be worth trying. A purer form of BtA would allow us to explore this possibility." (Editorial, "Botulism Toxin"

Lancet (1992) 3:1508-09 at p.1508). Again, the proposed solution to the problem of antibody formation was to increase the purity of the toxin preparations injected into patients.

Notably, neither of these reviews discloses nor suggests the possibility of using 1) a human-derived botulism immune globulin; 2) which immune globulin would be injected intravenously; 3) in order to prevent the unwanted side effects of toxin diffusion and antitoxic antibody formation in treated patients.

A detailed discussion regarding the development of antibodies to botulinum toxin in toxin-treated patients was reported by Hatheway and Dang. (Hatheway, C.L., Dang, C., "Immunogenicity of the Neurotoxins of Clostridium botulinum" in Jankovic, J., Hallet, M. eds. Therapy with Botulinum Toxin, Marcel Dekker, New York, NY (1993, in press)) Eighty-eight patients in the U.S. were followed for one year after they began treatment with toxin, during which time the amount of toxin received ranged from 0 to 2550 units. At one year into treatment, 29 patients (33%) had developed neutralizing antibody against the toxin. The antibody-positive patients had received an average of 1051 t.u. of toxin, while the antibody-negative patients had received an average of 301 t.u., again suggesting a dose-response effect in the induction of antibody. This dose-response possibility was borne out when the patients were stratified according to dose received: <500 treatment units, 4% with antibody; 500-1000 treatment units, 45% with antibody; 1000-2000 treatment units, 83% with antibody; >2000 treatment units, 100% with antibody. In addition, Hatheway and Dang noted that continued treatment of patients who have subdetectable levels of antibodies might serve to boost the antitoxin titers above the minimum demonstrable level.

Patients who require botulinum toxin injections generally must have the injections repeated at regular intervals. Dose-response data, such as that of Hatheway and Dang, suggest that as the duration of currently practiced botulinum toxin treatment is extended, more patients will develop antibody and thereby lose the therapeutic benefit of the toxin. Thus, in order to preserve the effectiveness of botulinum toxin as a therapeutic agent, a method is needed to prevent the development of these antibodies in patients injected with the toxin.

Although the problem of antibody development with botulinum toxin therapy has not been successfully addressed, other problems with the toxin therapy have been studied. It has been noted that patients injected with botulinum toxin have suffered complications due to the apparent diffusion of the toxin from the injected muscle(s) to adjacent muscles. For example, complications have included drooping eyelids (to the extent that vision is blocked), and difficulty with swallowing (to the extent that hospitalization was needed in order that a stomach feeding tube could be placed). (Jankovic, J., Brin, M.F., "Therapeutic Uses of Botulinum Toxin" N. Engl. J. Med. (1991) 324:1186-94; Schantz, E.J., Johnson, E.A., "Properties and Use of Botulinum Toxin and other Microbial Neurotoxins in Medicine" Microbiol Revs. (1992) 56:80-99) In certain clinical situations, such as with small or vitally-placed muscles, diffusion (or "leaking" of toxin) has limited the amount of toxin that could otherwise have been therapeutically injected, because of concern that such side-effects would develop. (Clinical Use of Botulinum Toxin. (*Reprinted from* NIH Conses. Dev. Conf. Consens. Statement 1990 Nov 12-14;8(8)); Jankovic, J., Brin, M.F., "Therapeutic Uses of Botulinum Toxin" N. Engl. J. Med. (1991) 324:1186-94;

Scott, A.B., "Clostridial Toxins as Therapeutic Agents" pp. 399-412 in Simpson, L.L., ed. Botulinum Neurotoxin and Tetanus Toxin, Academic Press, NY (1989); Scott, A.B., "Antitoxin reduces botulinum side effects" Eye (1988) 2:29-32)

An experimental attempt to overcome the side-effect of toxin diffusion into adjacent muscles was attempted by Scott. (Scott, A.B., "Antitoxin reduces botulinum side effects" Eye (1988) 2:29-32) Scott's effort utilized direct intramuscular injection of equine botulinum antitoxin. The antitoxin was injected into the toxin-treated muscles or into untreated adjacent eye muscles. At the time Scott did his clinical studies with antitoxin, the botulinum antitoxin was a horse-derived product available from Connaught Laboratories (Toronto, Canada).

In Scott's method of direct intramuscular injection of horse-derived antitoxin, Scott addressed only one of the fundamental problematic issues with botulinum toxin therapy: diffusion of toxin to adjacent muscles. Antibody development consequent to botulinum toxin use was neither contemplated nor addressed. Because of clinically observed weakness in muscles adjacent to those injected, Scott mentioned a theoretical possibility of intramuscular use of human-derived antitoxin: "A human-derived ATX [antitoxin] and the non-toxic large fragment of the toxin molecule to block unwanted toxin binding are additional related techniques to reduce side effects and to increase efficacy which we are pursuing and which avoid the theoretical risks of immunity or sensitisation [sic] to equine-derived proteins." (Scott, A.B., "Antitoxin Reduces Botulinum Side Effects" Eye (1988) 2:29-32 at p. 32) Again, however, this proposal for further study was set out in

the context of intramuscular antitoxin injection, with a goal of controlling toxin diffusion.

Although Scott might have been interested in using human botulism immune globulin (BIG) for intramuscular injections, he was unable to do so. At the time of Scott's study, the U.S. Army had the world's only supply of BIG. Also, at that time, it was not yet known that some patients injected therapeutically with botulinum toxin would develop antibodies to it. Scott's concern addressed only the possibility of development of antibodies to the equine botulism antitoxin.

Scott's approach was clinically unsatisfactory because it required the injection of additional muscles besides those targeted for the toxin (requiring additional physician/patient time and risk), and because the horse-derived botulinum antitoxin was a foreign protein capable of stimulating antibody production against itself when injected into humans. Hence, with repeated use, patients given the horse-derived antitoxin can be expected to develop antibodies against it and also to become refractory to its effects, just as some patients have become refractory to the effects of injected botulinum toxin. Of further concern, the horse-derived botulinum antitoxin is known to provoke severe allergic complications when used to treat patients with foodborne botulism: approximately one in eight such patients experienced anaphylaxis or serum sickness (i.e., allergic shock or kidney damage). (Black, R.E., Gunn, R.A., "Hypersensitivity Reactions Associated with Botulinal Antitoxin" Am. J. Med. (1980) 69:567-70)

In addition to its accepted usage for the treatment of strabismus and various dystonias, botulinum toxin has also been used to reduce facial wrinkles by temporarily weakening the underlying muscles. (Naik, G., Wall Street Journal (Friday, November 6, 1992) at p. B1)

If this cosmetic procedure finds widespread use, then based on the incidence of the dystonias in the general population, it is predicted that among the population cosmetically treated with botulinum toxin, some individuals will eventually experience the onset of a dystonia. For these patients to then be able to therapeutically benefit from injection of botulinum toxin, it is important that they would not have developed neutralizing antibodies against the toxin during their cosmetic treatment with it.

Passive Immunization:

Antibodies have been given to patients in order to achieve passive immunization. The antibodies may be obtained from human or animal donors who have recovered from an infectious disease or have been immunized. This antibody product can be either whole serum or fractionated concentrated immune (gamma) globulin, which is predominantly IgG. These antibodies can provide immediate protection to an individual deficient in such antibodies.

When antibodies are obtained from animals, the animal sera give rise to an immune response that leads to rapid clearance of the protective molecules from the circulation of the human recipient. Additionally, animal sera provide a risk of allergic reactions, particularly serum sickness or anaphylaxis.

With regard to human antibodies, special preparations of human immune globulin with a high titer of a specific antibody are available. These preparations are obtained by hyperimmunizing adult donors or by selecting plasma which was tested for a high specific antibody content. Although the side effects of human immune globulin are minimal, its intramuscular

administration is painful and, although rare, anaphylactoid reactions have been described.

Passive immunization has been carried out for infectious and noninfectious diseases. As an example of a noninfectious disease treated with passive immunization, Rh-negative persons are at risk of developing anti-Rh antibodies when Rh-positive erythrocytes enter their circulation. For Rh-negative women, this occurs regularly during a pregnancy with an Rh-positive fetus. Development of anti-Rh antibodies by a mother threatens all subsequent Rh-positive fetuses with erythroblastosis fetalis and death. This scenario can be prevented by administration of Rh immune globulin (RhIG) to the Rh-negative mother. RhIG is produced by having Rh-negative volunteers (originally men or nuns, because these women did not plan to have children) be injected with Rh-positive red cells to induce antibodies. Then, these volunteers are plasmapheresed to harvest the immune plasma, which is then processed into RhIG.

By use of RhIG, erythroblastosis fetalis is avoided in future Rh-positive fetuses. Passive immunization with Rh immune globulin (RhIG) suppresses the mother's normal immune response to any Rh-positive fetal cells that may enter her circulation. Passive immunization with RhIG may also protect in a nonspecific manner, analogous to the 'blocking' effect of high-dose IgG in ameliorating autoimmune diseases such as idiopathic thrombocytopenic purpura (ITP). With ITP, the beneficial blocking effect is thought to derive from the ability of the infused antibody to bind to receptors in the spleen and to prevent that organ from destroying the platelets to which the host's own "autoimmune" antibodies have become adherent. (Berkman, S.A., et al., "Clinical Uses of Intravenous Immunoglobulins" Ann. Int. Med. (1990) 112:278-292)

Passive immunization can be carried out in another context that relates to Rh isoimmunization. Rh isoimmunization may occur consequent to blood transfusion. Most transfusion reactions to Rh can be prevented by transfusing Rh-negative individuals with Rh-negative blood. Of the Rh antigens, the D antigen is a high-incidence, strongly immunogenic antigen, approximately 50 times more immunogenic than the other Rh antigens. Thus, when determining Rh status, transfusion blood is typed routinely for D, but not for other Rh antigens. However, immunization to other Rh antigens may occur even when Rh-negative blood is given to Rh-negative patients, since donor blood is not routinely typed for the non-D Rh antigens. Additionally, immunization and antibody formation to Rh antigens can occur in Rh-negative individuals due to transfusion errors. RhIG can be used to passively immunize and protect individuals from such situations. RhIG addresses a spectrum of Rh antigens because of the way it is made, utilizing red cells that contain an array of Rh antigens. Thus, the resulting RhIG is directed to various Rh antigens, in addition to the Rh D antigen.

Accordingly, Rh immunization can now be suppressed almost entirely if high-titer anti-Rh immunoglobulin (RhIG), available under the tradename Rhogam™ (Ortho Pharmaceuticals, Raritan, New Jersey), is administered within 72 hours of the time the potentially sensitizing dose of Rh-positive cells were given.

As is the case with RhIG administration to pregnant Rh-negative women, the protective mechanism by which RhIG administration prevents development of Rh antibodies in Rh-negative individuals is not clear. RhIG does not effectively block Rh antigen from immunoresponsive cells by competitive inhibition, since it is known that effective doses of RhIG do not cover all

Rh antigen sites on the fetal (or wrongly transfused) erythrocytes. Intravascular hemolysis with rapid clearance of erythrocyte debris by the reticuloendothelial system is also unlikely. Rather, after the Rh-positive cells are removed from the circulation, the RhIG-induced erythrocyte hemolysis is believed to be extravascular, primarily by phagocytic cells in the spleen and, to a lesser extent, the lymph nodes. The most likely therapeutic mechanism resulting from RhIG administration is a negative modulation of the primary immune response. It is believed that antigen-antibody complexes become bound to lymph node and splenic cells that have Fc receptors. These lymph node and spleen cells presumably then stimulate suppressor T cell responses, which subsequently prevent antigen-induced B cell proliferation and antibody formation.

Summary of the Invention

The present invention relates to a method to provide an adjunct to the therapeutic administration of a toxin, such as a neurotoxin. The method comprises providing a human-derived antitoxin, where the antitoxin corresponds to the administered toxin. Thereafter, the antitoxin is intravenously injected into the patient who received the corresponding toxin. In particular, the neurotoxin may be botulinum toxin. Additionally, the toxin used for therapeutic administration may be other neurotoxins such as *Clostridium baratii* type F-toxin, *Clostridium butyricum* type E-like toxin, or tetanus toxin. Furthermore, the antitoxin for the invention may be produced as monoclonal or polyclonal antibodies. Preferably, the antitoxin is botulism immune globulin, although antitoxin corresponding to any therapeutically administered toxin can be employed.

The present invention also comprises a method for treating a patient who has a neuromuscular disorder, whereby the treatment method comprises administering an amount of a toxin. Thereafter, a human-derived antitoxin is provided, where the antitoxin corresponds to the administered toxin. Then, the antitoxin is intravenously injected to the patient who received the toxin. For the method of treating, about 0.1 to 400 treatment units of botulinum toxin are administered during a treatment session. Generally, in this context, sufficient botulinum immunoglobulin is intravenously injected so as to neutralize about 10% to 90% of the injected toxin.

Detailed Description of the Invention

For the first time in the art a method is disclosed that prevents the development of antitoxin antibodies in patients treated with a toxin, such as a neurotoxin or the like, and prevents the unwanted side-effects, such as weakness of nontargetted muscles, due to the therapeutic administration of neurotoxin or the like.

Typically, the method comprises use of intravenously injected, human-compatible, human-derived antitoxin antibodies. By "human-compatible" is intended human-derived antibodies or antibodies that are not derived from a human source but which as provided have attendant features which allow them to avoid production of adverse effects, such as allergic reactions when administered to a human. Accordingly, by use of human-compatible antitoxin antibody, patients can continue to obtain the therapeutic benefits of a toxin therapy, such as neurotoxin therapy or the like, upon subsequent treatment.

The method of the invention relates to any toxin that is used as a therapeutic agent in human or animal disease, together with use of a corresponding

antitoxin. Intravenous injection of human-compatible antitoxin at an appropriate dosage and time prevents toxin that escapes an injection site from stimulating production of antitoxin antibodies by the patient's immune system.

Preferably, the method relates to the use of botulinum toxin. The method also includes the use of neurotoxins produced by other sources, for example, the botulinum F-like toxin of *Clostridium baratii*, and the botulinum E-like toxin of *Clostridium butyricum*, and any other such toxins as may be discovered in the future. This method also includes the use of human botulinum or botulinum-like antitoxins, or other human-derived antitoxins, that may be produced *in vitro* as polyclonal or monoclonal antibodies from cell cultures.

Accordingly, the method relates to tetanus toxin and other biological toxins (plant, animal and microbial, regardless of chemical structure or nature), should any of these be developed as therapeutic agents for use in human or veterinary medicine. Examples of such toxins include, but are not limited to, snake venom toxins, scorpion toxins and microbial toxins such as saxitoxin, neosaxitoxin, tetrodotoxin, brevitoxins, and ciguatoxin.

The method of preventing unwanted side-effects, such as production of endogenous antitoxin antibody, is based on the following three preferred principles:

1. Sufficient human-compatible antitoxin is intravenously injected to neutralize any corresponding toxin that escapes from the treatment site, so that the toxin does not stimulate the patient's immune system to produce endogenous antitoxin antibodies.
2. Intravenous injection facilitates that the antitoxin is dispersed throughout the

extracellular fluid, where it mixes with and binds any escaped toxin, before the toxin diffuses into nontargetted tissues.

3. The administration of antitoxin is advantageously delayed for a relevant period after therapeutic administration of corresponding toxin, so that the injected toxin can bind to the intended site(s).

EXAMPLES

Example 1: Use of Botulinum Toxin and Botulism Immune Globulin

In one embodiment, the invention includes use of human-compatible botulinum antitoxin antibodies injected intravenously to preclude the effect of any toxin that leaks or diffuses away from the site of injection. Use of human-derived botulinum antitoxin antibodies avoids the problems of allergic sensitization and antibody induction that occur with the horse-derived antitoxin.

Botulism Immune Globulin (BIG):

A new preparation of human-derived botulinum antitoxin recently came into existence as an Investigational New Drug (BB-IND-4283); this antitoxin is called Botulism Immune Globulin (BIG). Unlike the BIG made earlier by the U.S. Army, this new preparation of BIG was made in complete compliance with the requirements for licensure of biological products established by the U.S. Food and Drug Administration (FDA). Specifically, for human-derived immune globulins, the FDA requires that the source plasma be collected in an FDA-licensed plasmapheresis facility and that the source plasma be fractionated into immune globulin in an FDA-licensed facility that meets the standards of Good Manufacturing

Practice (GMP). BIG contains IgG antibodies that can neutralize botulinum toxin types A-E; these antibodies were obtained by plasmapheresis of botulinum toxoid-immunized volunteers. (Arnon, S.S., "Clinical Trial of Botulism Immune Globulin" pp. 485-90 *in* DasGupta, B.R., ed. Botulinum and Tetanus Neurotoxins: Neurotransmission and Biomedical Aspects, Plenum Press, NY (1993, in press))

Botulism Immune Globulin Intravenous (Human) is a sterile lyophilized powder of immunoglobulin G (IgG), stabilized with 5% sucrose and 1% Albumin (Human). It contains no preservative. The purified immunoglobulin was derived from pooled adult human plasma from persons immunized with pentavalent (ABCDE) botulinum toxoid, who were selected for their high titers of neutralizing antibody against botulinum neurotoxin types A,B,C,D, and E. All donors were tested and found negative for antibodies against the Human Immunodeficiency Virus (HIV), the hepatitis B and C viruses, and the HTLV-I virus. In addition, each individual unit of donated immune plasma was tested and found negative for antibody against the HIV and hepatitis B viruses.

The pooled plasma was fractionated by ethanol precipitation of the proteins according to Cohn Methods 6 and 9, modified to yield a product suitable for intravenous administration. Cohn methods 6 and 9 are known to be capable of inactivating the AIDS (HIV) virus. When reconstituted with Sterile Water for Injection, USP, each milliliter contains 50±10 mg of immunoglobulin, primarily IgG, and trace amounts of IgA and IgM; 50 mg of sucrose; 10 mg of Albumin (Human). The reconstituted solution appeared colorless and translucent.

Toxin Treatment:

The method of the invention includes the use of botulinum toxin types for which human-derived antitoxin

antibody presently exists (A-E), as well as for human-derived antitoxin antibody to types F and G, and for human-derived antitoxin antibody for any botulinum toxin types that may be discovered in the future.

The treatment dose of botulinum toxin which is injected into a patient varies with the size and location of the muscle(s) to be treated; the dose of BIG which is injected intravenously varies accordingly. For adults, the therapeutic dose of botulinum toxin which is injected per session ranges from as little as about 0.1 treatment unit (t.u.) of botulinum toxin (e.g., for cosmetic eye wrinkle use), and in certain circumstances, to as much as about 400 t.u. (e.g., in a very large dystonia patient, such as a professional football player or sumo wrestler). The desired amount of BIG that is injected is an amount sufficient to neutralize about 10% to 90% of the injected toxin. The amount of toxin neutralized can vary depending on such criteria as the disease and age, size and the like of the patient treated so that the amount may not be the same for every disease or every patient; the optimal ratio is determined based on clinical experience possessed by those of ordinary skill in the art. The optimal ratio depends on the proportion of injected toxin that leaks from the injected muscle(s); this proportion is not the same for all muscles because of the vastly different sizes of muscles injected in different diseases, as is appreciated and known to those of ordinary skill in the art. However, it is understood that anywhere from 10% to 90% of injected toxin may be able to leak from the injection site, and hence, enough BIG is provided to neutralize this amount of toxin. Additionally, BIG which is injected intravenously distributes itself over the entire volume of extracellular fluid (i.e., the interstitial and intravascular fluid compartments). It is recognized that

in some clinical situations a locally higher concentration of BIG is called for (e.g., when large amounts of toxin are injected into dystonic neck muscles); this is taken into account when determining the amount of BIG which is provided.

BIG is calibrated in International Units (IU), whereas botulinum toxin is calibrated in treatment units (t.u.). The relation between the two is that, by definition, 1 International Unit (IU) of BIG neutralizes 10,000 treatment units (t.u.) of type A botulinum toxin. Therefore, the amount of BIG needed by a patient in a single intravenous injection to accompany an intramuscular treatment with botulinum toxin is generally be between 1×10^{-6} to 3.6×10^{-2} International Units of BIG.

Preferably, the desired amount of BIG is injected intravenously between about 2 and 24 hours after the intramuscular injection of botulinum toxin. An initial 4-hour delay is preferred, so as to provide the toxin with sufficient time to bind at the intended sites in the treated muscle. The time range of post-toxin injection of BIG is to permit necessary clinical latitude appropriate to individual patient circumstances, as the optimal time interval can vary depending on the patients and the disease conditions. However, if BIG is not administered within this 2-24 hour time interval, yet still in accordance with the invention, BIG can be given up to 72 hours to prevent the induction of antitoxin antibodies. Late administration of BIG may not fully prevent the weakening of muscles adjacent to the toxin injection site, depending on the local anatomy and the amount of toxin which is injected.

Preferably, only a single intravenous injection of BIG is needed at each treatment session because the half-life of human immunoglobulin in humans is

approximately 30 days. (Ochs, H.D., et al., "Survival of IgG Subclasses Following Administration of Intravenous Gamma-Globulin in Patients with Primary Immunodeficiency Diseases," pp. 77-85 *in* Morell, A., Nydegger, U.E., eds., Clinical Use of Immunoglobulins, Academic Press, London (1986); Mankarious S., et al., "The Half-Lives of IgG Subclasses and Specific Antibodies in Patients with Primary Immunodeficiency Who Are Receiving Intravenously Administered Immunoglobulin," J. Lab Clinc Med. (1988) 112:634-40) The half-life of BIG correlates with a further consideration when establishing the dosage of BIG. The effects of intramuscular injected toxin typically begin to wear off about 4 months after treatment, whereupon retreatment typically becomes necessary. Consequently, at the time of toxin retreatment, it is important that before retreatment, the BIG level in the circulation has declined to sub-clinical significance; otherwise, residual BIG serves to partially block the effect of the next toxin treatment. For this reason, and as readily appreciated by those of ordinary skill in the art, it is important to give the patient only the amount of BIG indicated for the specific amount of toxin administered at each treatment session.

There is variation among patients in their duration of illness before coming to treatment, in the severity of their illnesses, in the size of their muscle(s) needing toxin injection, in their individual sensitivity to equivalent amounts of injected toxin, and hence, in their overall response to treatment with toxin, as is known and appreciated by those of ordinary skill in the art. For this reason, the amount of toxin which is injected into a particular patient with a particular problem at a particular time is a clinical decision to be determined by the attending physician, based on information known to those of ordinary skill in the art.

Since the amount of toxin that either binds at or leaks from the treatment site varies with the clinical circumstances, some patients treated with both toxin and BIG have a diminished response to the injected toxin, because some portion of the toxin is neutralizable at the time the BIG is injected. Diminution in the effect of injected toxin may be overcome by increasing the dose of toxin (or decreasing the dose of BIG) during treatment sessions or by repeating the toxin and BIG injections at shorter intervals, as appreciated by those of ordinary skill in the art.

Example 2: Use of *Clostridium perfringens* Kappa Toxin and Corresponding Antitoxin

Collagen is the major structural protein in the body, and a main component of connective and fibrous tissue. Accordingly, collagen constitutes approximately 25% of all body protein.

The obligate anaerobic bacterium *Clostridium perfringens* produces numerous "virulence factors," some of which are lethal to mammals and therefore referred to as toxins (Smith, L.D.S., "Virulence factors of *Clostridium perfringens*." Rev. Infect. Dis. (1979)1:254-60; McDonel, J.L., "*Clostridium perfringens* Toxins (Type A,B,C,D,E)." Pharmac. Ther. (1986) 10:617-55). One of these "virulence factors," the kappa toxin, is a protein molecule and enzyme that digests collagen (a "collagenase").

The collagenase (kappa toxin) produced by *C. perfringens* is used to treat patients with excessive fibrous connective ("scar") tissue. Because collagen is a component of many body tissues, it is particularly important that injected collagenase ("kappa toxin") not be able to diffuse away from the site of therapeutic administration and affect adjacent tissues. Further,

therapeutic use of kappa toxin induces the patient's body to produce antitoxin. This antitoxin corresponding to the kappa toxin decreases the treatment efficacy of the kappa toxin. To overcome the problems of toxin diffusion and induction of antitoxin antibodies, a method in accordance with the invention is carried out for the treatment of several conditions. Treating patients undergoing kappa toxin therapy with the provision of an appropriate antitoxic antibody directed against kappa toxin enables these therapeutic considerations to be met.

For example, a tendency to produce excessive connective ("scar") tissue in response to traumatic injury is particularly prevalent among some persons of African descent. This tendency to produce excessive collagen is genetically determined and is present throughout an effected person's life. This excessive formation of scar tissue occurs in response to otherwise trivial injury and may become quite cosmetically objectionable to the patient. On the skin these abnormal accumulations of fibrous tissue are termed "keloids"; typically they are large, raised, unsightly lesions.

Kappa toxin is used to treat the formation of excessive connective tissue in effected individuals. These methods eliminate connective tissue accumulations or reduce their size, and are generally repeated over the life of the patient. Due to repeated exposure to the toxin, antibodies to the toxin develop in some patients. These antibodies reduce the efficacy of the treatment. Hence it is important that a patient not develop antibodies against kappa toxin when the toxin is used for this purpose.

Also, certain chronic diseases are characterized by proliferation of connective tissue which then becomes injurious to the patient. Rheumatoid arthritis is such a disease, whereby chronic inflammation

of an effected joint capsule leads to excessive connective tissue formation, and consequently, to enlarged, immobile joints. Kappa toxin is used to treat this connective tissue formation. Because rheumatoid arthritis is a chronic disease, these patients also require repeated treatments with kappa toxin to reduce or eliminate the unwanted connective tissue proliferation. Due to repeat exposure to kappa toxin, the patients can develop antibodies to the toxin. These antibodies reduce the efficacy of the treatment. Because this treatment generally must continue for the duration of the disease (i.e., the patient's remaining lifetime), it is important that the patient not develop antibodies against kappa toxin when it is used for this therapeutic purpose.

Accordingly, human-compatible, human-derived antibodies to kappa toxin are obtained by injecting volunteers with kappa toxoid, made by treating kappa toxin with formalin in accord with standard procedures for preparing toxoids of protein molecules. Thereafter, appropriate therapeutic administration, in accordance with parameters appreciated by those of ordinary skill in the art, of the anti-kappa-toxin antibody protects the patient against the unwanted development of endogenous antibody, antibody that would result in insensitivity to further treatment with kappa toxin. Also, provision of anti-kappa-toxin antibody protects against diffusion ("leakage") of the collagenase from its injection site into other tissues.

Example 3: Use of Cobra Neurotoxin and Corresponding Antitoxin

Cobra neurotoxin is used in the treatment of spasmodic dystonias. Use of cobra neurotoxin in this manner is a further example in which exogenous antitoxin antibodies are used to protect patients from becoming

refractory to therapeutically injected toxin, as a consequence of the patient developing antibodies against the toxin. Use of cobra neurotoxin offers dystonia patients a additional means, in addition to use of botulinum toxin, for treating their disease.

Cobra neurotoxin is a small (MW 6949 daltons) basic protein that, like botulinum toxin, produces flaccid paralysis by its action at the neuromuscular junction. Unlike botulinum toxin which acts pre-synaptically, however, cobra neurotoxin acts post-synaptically in a manner pharmacologically similar to *d*-tubocurarine (Lee CY. "Mode of Action of Cobra Venom and its Purified Toxins" *in* Simpson, L.L., ed., *Neuropoisons: their pathophysiological actions*, pp. 21-70. (Plenum Press, NY, 1971)).

Cobra neurotoxin is a much smaller molecule than crystalline botulinum toxin. Consequently, it diffuses away from the injection site more readily than botulinum toxin. Since it is a foreign protein, cobra neurotoxin that escapes the injection site can, both, stimulate an unwanted antibody response by the patient, and weaken muscles adjacent to the treated muscles. For these reasons it is particularly advantageous that patients treated with cobra neurotoxin receive antitoxin antibodies to prevent the occurrence of these unwanted complications of treatment.

Human-compatible antibodies to cobra neurotoxin (cobra antitoxin) are produced according to known methodologies. The cobra antitoxin is administered in an appropriate regimen, according to parameters appreciated by those of ordinary skill in the art, and prevents development of endogenous antibodies by the patient. Were the patient to have developed antibodies to the cobra toxin, treatment efficacy would be diminished. By

use of the human-compatible antibodies, continued efficacious treatment with the toxin occurs.

All publications and patent applications cited in this specification are incorporated by reference herein, as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention, that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.